Development of a Novel Canine Model of Ischemic Stroke: Skull Base Approach with Transient Middle Cerebral Artery Occlusion

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■ OBJECTIVE: **Although canine stroke models have several intrinsic advantages, establishing consistent and reproducible territorial stroke in these models has been challenging because of the abundance of collateral circulation. We have described a skull-base surgical approach that yields reproducible stroke volumes.**

■ METHODS: **Ten male beagles were studied. In all 10 dogs, a craniectomy was performed to expose the circle of Willis. Cerebral aneurysm clips were temporarily applied to the middle cerebral artery (MCA), anterior cerebral artery (ACA), posterior cerebral artery, and/or ophthalmic artery (OA) for 1 hour, followed by cauterization of the distal MCA pial collateral vessels. Indocyanine green angiography was performed to assess the local blood flow to the intended area of infarction. The dogs' neurologic examination was evaluated, and the stroke burden was quantified using magnetic resonance imaging.**

■ RESULTS: **High mortality was observed after 1-hour clip occlusion of the posterior cerebral artery, MCA, ACA, and OA ($n = 4$). Without coagulation of the MCA collateral vessels, 1-hour occlusion of the MCA and/or ACA and OA yielded inconsistent stroke volumes ($n = 2$). In contrast, after coagulation of the distal MCA pial collateral vessels, 1-hour occlusion of the MCA, ACA, and OA yielded consistent territorial stroke volumes ($n = 4$; average stroke volume, 9.13 $\pm$ 0.90 cm^3; no surgical mortalities), with reproducible neurologic deficits.**

■ CONCLUSION: **Consistent stroke volumes can be achieved in male beagles using a skull base surgical approach with temporary occlusion of the MCA, ACA, and OA when combined with cauterization of the distal MCA pial collateral vessels.**

INTRODUCTION

Worldwide, 15 million people will be afflicted by stroke every year, with one third dying and an additional one third left with a permanent disability. Therapeutic paradigms to mitigate the neurologic injuries associated with stroke have largely been developed from murine and rodent models.[1-4] Although these powerful experimental models have yielded valuable insights into stroke, therapies that showed enormous promise in these models have failed in clinical translation.[1,2,5-9] An expert panel was assembled by the National Institutes of Health to address these failures.[10] This panel recognized that any single experimental model might not necessarily recapitulate the human pathophysiology of stroke. Because no consensus has been reached regarding the "optimal" model for stroke, the panel recommended testing of potential therapies in multiple species and in animals with gyrencephalic

Key words
- Animal stroke
- Canine
- Canine stroke
- Experimental stroke
- Middle cerebral artery
- Stroke model

Abbreviations and Acronyms
ACA: Anterior cerebral artery
CMRR: Center for Magnetic Resonance Research
CSCARS: Canine stroke clinical assessment rating scale
ICA: Internal carotid artery
ICG: Indocyanine green
IM: Intramuscularly
IV: Intravenously
MCA: Middle cerebral artery
MRI: Magnetic resonance imaging
OA: Ophthalmic artery
PCA: Posterior cerebral artery
ROI: Region of interest

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brains.[10] In addition to harboring vasculature patterns that are more similar to human subjects, the larger caliber of the blood vessels in these large animal gyrencephalic models affords an opportunity to deliver the therapy through endovascular interventions[11-13] or combining therapy with these interventions.[11,12]

In this context, the development of a consistent and reproducible territorial stroke model in gyrencephalic animal models would serve an essential need in stroke investigations. Pertinent models in this regard include nonhuman primates, dogs, swine, rabbits, and cats. In general, nonhuman primates, dogs, and swine have been preferred over rabbits and cats because of the size of the peripheral and intracranial blood vessels in the former, which enable testing of endovascular devices used to treat human stroke. Although swine have frequently been used for testing thrombectomy devices in peripheral vessels, they are poor models of intracranial stroke because the rete mirabile at the base of the skull precludes intracranial endovascular access. The nonhuman primate models are associated with the risk of herpes B virus transmission, which is highly pathogenic, even deadly,[14,15] to humans. Moreover, nonhuman primate models are laborious and cost-prohibitive when considering larger sample size studies. In this context, dogs have emerged as an attractive model for stroke studies.

Because of the complexity of the collateral circulation and normal anatomic variation in cerebral vasculature (also seen in human subjects), a major challenge in developing canine models of stroke involve the creation of reproducible stroke volumes. For instance, the stroke volume resulting from the occlusion of the middle cerebral artery (MCA) can vary $\leq$100-fold in a canine model.[16] The aim of the present study was to develop a canine model of stroke that was highly reproducible with low mortality. Furthermore, we were interested in developing a model of stroke that could be used to study therapies targeting the ischemic brain such as cellular therapies, neuroplasticity, and neuroregenerative methods.

We report the development of a skull base surgical approach that combines temporary occlusion of the proximal MCA with permanent cauterization of the MCA collaterals in a canine stroke model. Cauterization of the distal collateral vessels is novel and, we believe, critical to developing a reproducible stroke model. The approach yielded a reproducible stroke volume and minimized the mortality related to the procedure. We also compared the merits of this model relative to other available gyrencephalic models.

METHODS

Surgical Procedures

The University of Minnesota institutional animal care and use committee had approved all protocols for the studies, which were performed with the close oversight and collaboration with the veterinary staff at the University of Minnesota and Research Animal Resources.

Adult male beagles (weight, 8–11 kg; ~2 years old) were purchased from Ridglan Farms, Inc. (Glenbeulah, Wisconsin, USA). All the dogs were housed indoors at a University of Minnesota Research Animal Resources facility under standard conditions. Twelve hours before the surgery, the dogs were orally administered 2.5 mg/kg phenobarbital as an antiepileptic and were fasted with free access to water. At the procedure, each dog was sedated with acepromazine, 0.05–0.2 mg/kg, administered intramuscularly (IM), and transported to the operating room. Two 18-gauge intravenous (IV) catheters were placed in either the forelimbs or hind limbs depending on vein accessibility. The dogs then received IV propofol, 4–6 mg/kg, were intubated, and maintained under general anesthesia using isoflurane (1.5%–2.5%) and 100% oxygen for the duration of the procedure. The dogs were positioned on a radiolucent surgical table on a heating blanket to maintain body temperature (range, 99.5°–102.5°F). Before beginning the procedure, eye lubricating ointment was applied to both eyes, the eyes were closed, and the dogs received a 30-mg/kg IV dose of cefazolin (Ancef [GlaxoSmithKline, Brentford, United Kingdom]). Throughout the procedure, the vital signs, including heart rate, electrocardiogram, respiration, blood pressure, oxygen saturation level, and end-tidal carbon dioxide (range, 32–38 mm Hg) were monitored.

After the dog had been anesthetized, the right frontal region of the head and both groins were clipped, prepared with alcohol and betadine, and draped for surgery. Using a no. 10 scalpel blade, a curvilinear incision (1–2.5-in. long) was made 1 cm above the level of the right zygoma. The skin was then reflected inferiorly and held in place by a suture. Hemostasis was obtained using bipolar and monopolar cautery. The zygomatic arch was identified and removed using a pneumatic drill (Anspach EMAX [DePuy Synthes, Palm Beach Gardens, Florida, USA]). Bleeding from the bone was controlled with bone wax (Ethicon, San Angelo, Texas, USA). The temporalis muscle was then incised in a linear fashion, down to the underlying cranium, using monopolar cautery. After mobilizing the temporalis muscle off the cranium using a periosteum, the muscle was held in place with fishhook retractors. At this point, the coronoid process of the mandible obstructed the view of the skull base. Thus, a small portion of the coronoid process was removed using a bone rongeur to expose the skull base. A craniectomy (2 cm $\times$ 2 cm) was performed using the pneumatic drill over the frontal and temporal lobes. The craniectomy was extended inferiorly to completely expose the base of the middle temporal fossa. Hemostasis was achieved with bone wax and bipolar cautery.

A neurosurgical operating microscope (model 5–1000 [Haag-Streit, Wedel, Germany]) was used for the remainder of the procedure. After performing the craniectomy, we opened the dura in a cruciate fashion using a Metzenbaum scissors. The frontal and temporal lobes were then gently retracted with a small brain retractor. The arachnoid at the base of the brain was incised using an 18-guage needle attached to an insulin syringe. This exposed the blood vessels constituting the circle of Willis. Cerebrospinal fluid was aspirated to relax the brain. Using microscopic dissection techniques, we exposed the internal carotid artery (ICA), MCA, posterior cerebral artery (PCA), and anterior cerebral artery (ACA), which in the dog is an azygous variant. Cerebral aneurysm clips (L-series [Peter Lazic, Tuttlingen, Germany]) were then temporarily applied to the ICA, MCA, PCA, or ACA or a combination of these vessels for 1 hour. In some dogs, the distal collateral branches to the MCA were coagulated. The local blood flow to the intended area of infarction was then evaluated with either IV indocyanine green (ICG; 1.0 mg/kg) imaged using the

ICG filter on the surgical microscope or with cerebral angiography.

After 1 hour of temporary occlusion, the aneurysm clips were removed, restoring the blood flow to the respective blood vessels. The surgical site was irrigated with saline to ensure no bleeding was present. The skin was closed with a running 2–0 nylon suture. The dog was then extubated and taken to the recovery area.

Postoperatively, the dogs were observed closely until they could stand on all 4 legs, regulate their body temperature, and had begun to eat and drink water. Typically, this required direct observation for 12–24 hours. The dogs continued to receive twice-daily phenobarbital (2.5 mg/kg) for 14 days.

Cerebral Angiography

Angiography was performed with the dogs intubated and anesthetized for the stroke surgical procedure. In brief, the left common femoral artery was selected for the initial vascular access. If access on the left could not be achieved, the right vessel was used. The left common femoral artery was identified through a surgical cutdown procedure, and vessel loops were placed to isolate a segment to create a small arteriotomy. A flexible, soft-tipped wire was advanced through the arteriotomy into the common femoral artery and a 5F, short, arterial sheath was passed over the wire.

Through the 5F sheath, a 5F, angled tip glide catheter (Terumo, Somerset, New Jersey, USA) was advanced over a 0.35-in. guide wire. This was advanced into the descending aorta and used to selectively catheterize the right and left common carotid arteries and vertebral arteries. Once the vessels had been selectively catheterized, the wire was removed, and selected arterial injections were performed. Angiograms were performed by manual injection of Omnipaque 300 contrast (GE Healthcare, Chicago, Illinois, USA) into the selected vessels and imaged using digital subtraction angiography with a GE OEC 9800 C-arm (GE Healthcare). After the final images had been obtained, the 5F catheter was removed. The arterial sheath was then removed, and the arteriotomy was closed primarily using 6–0 sutures. The skin incision was closed primarily using 2–0 silk suture in the dermis and a running nylon stitch in the skin.

Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) was performed at either the University of Minnesota Medical Center or University of Minnesota Center for Magnetic Resonance Research (CMRR) 1 day after stroke. At the University of Minnesota Medical Center, the MRI studies were obtained using a 3T whole-body MRI scanner (Trio Trim [Siemens Medical Solutions, Erlangen, Germany]) using an 8-channel, receive-only head coil (Siemens Medical Solutions). At the CMRR, the MRI studies were obtained using a 7T whole-body MRI scanner (MAGNETOM 7T, 90-cm bore, actively shielded [Siemens Medical Solutions]) with a 32-channel, receive-only head coil (produced at CMRR). The MRI sequences included T1-weighted, T2-weighted, fluid-attenuated inversion recovery, diffusion-weighted imaging, apparent diffusion coefficient, and gradient echo sequences.

For 12 hours before MRI, all the dogs were fasted with free access to water. At the procedure, each dog was sedated with acepromazine (range, 0.05–0.2 mg/kg IM). Two 18-gauge IV catheters were placed in either the forelimbs or hind limbs depending on vein accessibility. Anesthesia was induced with propofol (4–6 mg/kg IV to effect), and the dogs were intubated. During MRI scanning, the dogs received assisted ventilation using the rebreathing bag, and sedation was maintained with IV boluses of propofol. The vital signs, including heart rate, electrocardiogram, respiration, blood pressure, and oxygen saturation level, were monitored.

Behavioral Assessment

Behavior studies were conducted using a neurologic assessment adapted from Boulos et al.[17] performed by a dedicated veterinary staff. The dogs were assessed in the Research Animal Resources facility after induction of stroke on postoperative days 1 and 7. The scorecard used for the neurologic assessment in our canine stroke model is presented in **Table 1**. In brief, the dogs were assessed for consciousness, their ability to stand, presence of cerebellar ataxia, unilateral vestibular ataxia, bilateral vestibular ataxia, hemiparesis, paraparesis, proprioception, placing response (nonvisual and visual), tremor, auditory response (ipsilateral and contralateral side), and their ability to ambulate and vocalize. Normal neurologic examination findings would result in a score of 0 of 53 and the worst examination findings would result in a score of 53 of 53.

Euthanasia

The dogs were sedated with acepromazine (range, 0.05–0.2 mg/kg IM). An IV catheter was placed, and Beuthanasia solution (range, 87–90 mg/kg [Merck Animal Health, Madison, New Jersey, USA]) was administered. After cardiac arrest, a midline thoracotomy was performed, and the left heart ventricle was perfused with heparinized saline. Simultaneously, the right atrium of the heart was punctured, allowing egress of venous blood. Saline perfusion continued until only saline was seen from the right atrium, indicating that all the blood has been replaced by saline. At this point, 4% paraformaldehyde was infused into the left ventricle. To minimize the amount of paraformaldehyde required, we placed a Kelly clamp on the descending aorta such that only the upper extremities and brain were fixed with paraformaldehyde.

Immunohistochemistry

The brains were removed through a large craniectomy made with a pneumatic drill. Once removed, the brains were cut in the coronal plane into ~2-cm-thick slices and embedded in paraffin. The paraffin-embedded brain blocks were cut into 20-μm-thick sections using a microtome (American Optical 860 sliding microtome Buffalo, New York, USA). Tissue sections were mounted on gelatin-coated glass slides (ESCO 2-in. × 3-in. glass slides [Electron Microscopy Sciences, Hatfield, Pennsylvania, USA]) and stained with hematoxylin and eosin. In brief, the sections were rehydrated in hematoxylin solution for 40 minutes. They were then washed in tap water for 5 minutes and then immersed in 70% ethanol containing 1% hydrochloric acid for 5 seconds. They were again washed in tap water and stained with eosin for 10 minutes. This was followed by an additional wash in tap water, dehydration, and cover slip.

Table 1. Canine Stroke Clinical Assessment Rating Scale

Assessment Parameter and Description	Score
Stance	
Broad-based stance	1
Normal stance	0
Cerebellar ataxia	
Severe gait dysmetria	3
Moderate gait dysmetria	2
Modest gait dysmetria	1
Normal gait	0
Unilateral vestibular ataxia	
Leaning and falling to 1 side	
Severe leaning and falling to 1 side	3
Moderate leaning and falling to 1 side	2
Modest leaning and falling to 1 side	1
No leaning or falling	0
Head tilt	
Severe head tilt	3
Moderate head tilt	2
Modest head tilt	1
No head tilt	0
Nystagmus	
Severe nystagmus	3
Moderate nystagmus	2
Modest nystagmus	1
No nystagmus	0
Bilateral vestibular ataxia*	
Severe	3
Moderate	2
Modest	1
None	0
Hemiparesis	
Severe	3
Moderate	2
Modest	1
None	0
Paraparesis	
Severe	3
Moderate	2
Modest	1
None	0
Proprioceptive positioning†	
Right forepaw	
Severe delay	2
Moderate delay	1
Normal	0
Left forepaw	
Severe delay	2
Moderate delay	1
Normal	0
Right hind paw	
Severe delay	2
Moderate delay	1
Normal	0
Left hind paw	
Severe delay	2
Moderate delay	1
Normal	0
Placing response, nonvisual‡	
Right forepaw	
No response	1
Normal response	0
Left forepaw	
No response	1
Normal response	0
Placing response, visual§	
Right forepaw	
No response	1
Normal response	0
Left forepaw	
No response	1
Normal response	0
Tremor	
Severe	3
Moderate	2
Modest	1
None	0
Auditory response	
No response to noise	1
Normal orientation to noise	0
Auditory response	
Ipsilateral side	

Continues

Table 1. Continued

Assessment Parameter and Description	Score
No response	1
Normal orientation	0
Contralateral side	
No response	1
Normal orientation	0
Best ambulation attempt	
No movement	8
Unable to right self but moves	7
Able to right self	6
Unable to stand	5
Stands with assistance	4
Stands without assistance	3
Circles but falls to side	2
Circles	1
Normal	0
Vocalization	
None	2
Howls or grunts	1
Normal	0
Consciousness	
Does not awaken	3
Awakens to noxious stimulus	2
Awakens to minimal stimulation	1
Awake and interactive	0
Subtotal score for neurologic examination	0—53 of 53

The top of the form contains space to include the dog identification number, the dog's age, and the date of testing.
*Dog will exhibit side-to-side head movements, be reluctant to move, and maintain a crouched position.
†Invert the paw, placing the dorsal surface in contact with ground—the dog should immediately place the foot in the normal position.
‡Cover the dog's eyes, pick up the dog, and move it toward the edge of the table; when the paw touches the table edge, the dog should reflexively place the paw on the table surface.
§Same procedure as for the nonvisual task, but the eyes are allowed to remain open; the dog should reflexively extend the paw to the table surface before touching the edge.
(Adapted, with permission, from Boulos AS, Deshaies EM, Dalfino JC, Feustel PJ, Popp AJ, Drazin D. Tamoxifen as an effective neuroprotectant in an endovascular canine model of stroke. *J Neurosurg.* 2011;114:1117-1126.)

Volumetric Analysis

MRI volumetric analysis was performed on T2-weighted brain MRI scans obtained 24 hours after the stroke. MRI DICOM (Digital Imaging and Communications in Medicine) images were imported into an OsiriX (Pixmeo SARL, Bernex, Switzerland) DICOM viewer on an Apple iMac computer (Apple Inc., Cupertino, California, USA). The T2-weighted signal associated with the infarct was identified and outlined on each axial slice using a free-hand region of interest (ROI) tool, which gives the area enclosed by each ROI. The areas were summed and multiplied by the slice thickness and the number of slices on which the infarcted tissue was visible to generate the volume of infarcted tissue: $V = (\Sigma A) \times T \times N$, where V is the volume of infarcted tissue, ΣA is the sum of the areas (A) of the ROIs, T is slice thickness, and N is the number of slices on which the ROIs were drawn. The volumes from the different dogs were then averaged and the standard deviation was calculated.

RESULTS

Temporary MCA, ACA, and OA Occlusion

In our first experiment, we performed a frontotemporal craniectomy on an adult beagle (see the Methods section) and isolated the MCA through a skull base approach. We applied a cerebral aneurysm clip to the MCA (proximal to the lenticulostriate vessels) for 1 hour ($n = 1$). The clip was then removed and the incision closed (Figure 1A). No evidence of infarct was observed on the MRI studies taken 1 day after the procedure (Figure 1A′), and the dog had remained neurologically normal using the Canine Stroke Clinical Assessment Rating Scale (CSCARS; Table 1).

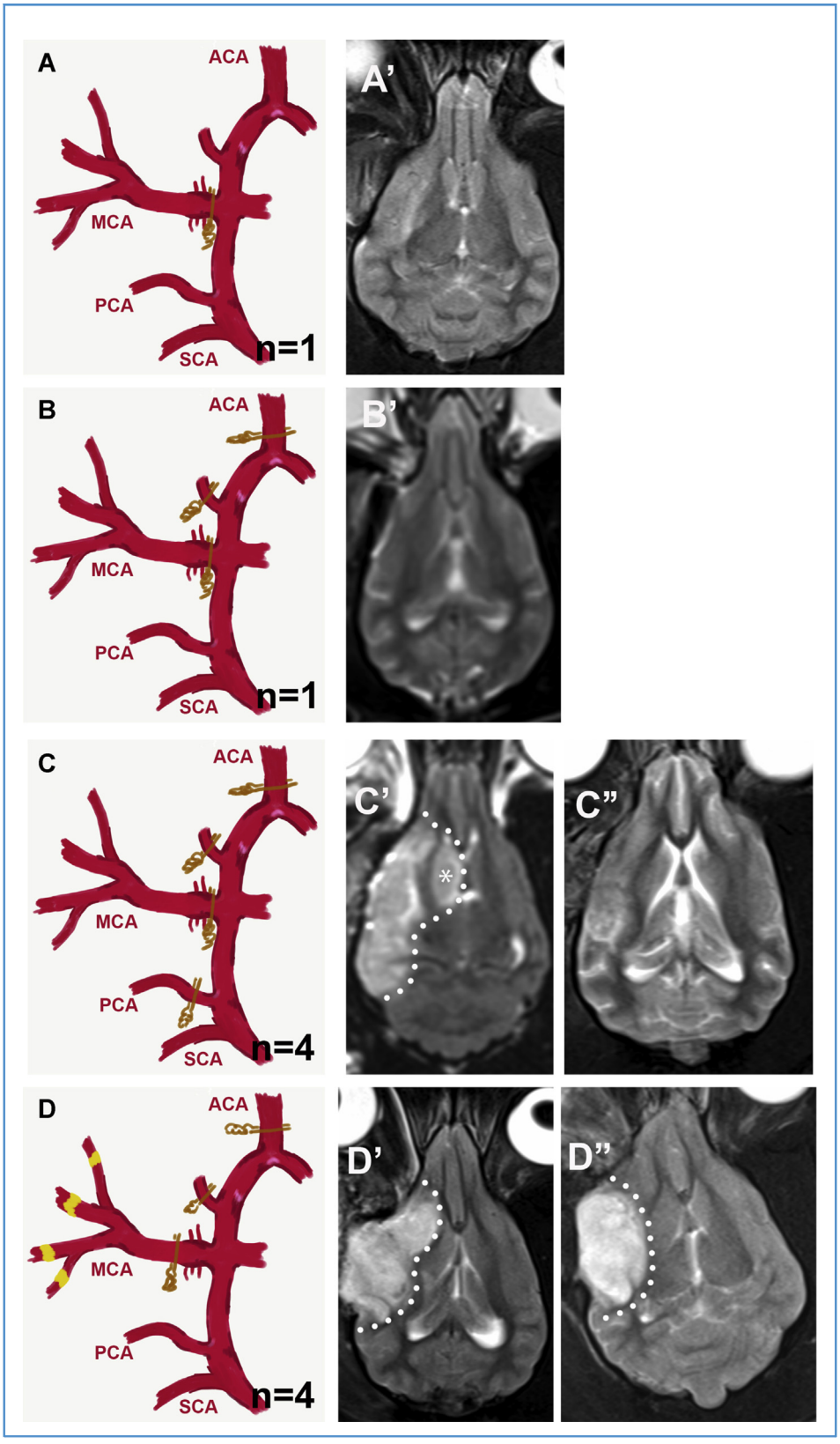
We next occluded the MCA, proximal to the lenticulostriate vessels, and the ophthalmic artery (OA) and the anterior cerebral artery (ACA) ($n = 1$) through temporary clip application for an hour (Figure 1B). Again, subsequent clinical assessment did not reveal evidence of neurologic injury, and MRI showed no evidence of cerebral infarct (Figure 1B′).

Temporary MCA, ACA, Ophthalmic Artery, and PCA Occlusion

We next performed temporary occlusion of the MCA (proximal to the lenticulostriate vessels), ophthalmic artery (OA), ACA, and PCA (Figure 1C). The procedure was performed in 4 dogs. Two of these dogs experienced significant hemodynamic or thermal dysregulation, ultimately developing cardiac arrest within 24 hours of surgery. Necropsy of these 2 dogs revealed a large territorial stroke, including the hypothalamus. In the 2 surviving dogs, MRI taken 1 day after craniectomy showed a large territorial infarct in 1 dog (Figure 1C′) and a small cortical infarct in the other (Figure 1C″). The former dog had a severe neurologic deficit and died soon after the MRI, and the latter dog exhibited only a minor neurologic deficit.

Temporary MCA, ACA, and OA Occlusion Combined with Cauterization of Distal MCA Collateral Vessels

The results from the previous experiments suggested significant variation in the perfusion contribution of collateral circulation during temporary clipping of the major intracranial vasculature. These variations constituted a major challenge to the establishment of a consistent and reproducible stroke model. Additionally, the hemodynamic and/or thermal dysregulation seen in some of the dogs was likely associated with hypothalamic infarction from lenticulostriate vessel occlusion. To address these issues, we opted to coagulate the collateral blood vessels to the distal MCA territory, in addition to the temporary occlusion of the MCA (distal to the lenticulostriate vessels), ACA, and OA (Figure 1D).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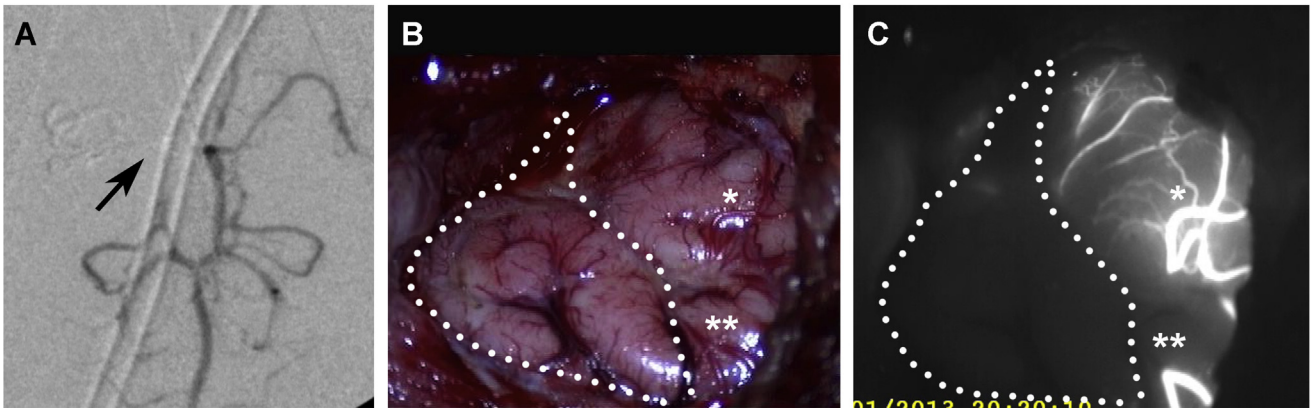

Figure 2. Intraoperative images depicting stroke. Images obtained during surgery in procedure D, in which the middle cerebral artery (MCA) distal to the lenticulostriate vessels, anterior cerebral artery (ACA), and ophthalmic artery (OA) between the MCA and ACA, had been temporarily occluded with aneurysms clips and the distal MCA collateral connections had been coagulated. Digital subtraction cerebral angiogram after injection with contrast in the basilar artery. Vessel clips applied to the 3 vessels can be seen. Contrast filled the circle of Willis. (**A**) A paucity of contrast is evident in the MCA territory (*arrow*). (**B,C**) Simultaneous images taken through a neurosurgical microscope depicting the MCA territory of the cerebral cortex. (**B**) Bright field view of the cerebral cortex with coagulated collateral vessels (*asterisk* and *double asterisks*). (**C**) Indocyanine green (ICG) fluorescent image depicting ICG in patent cortical vessels. Area of ischemia is evident by the paucity of ICG fluorescence (*dotted line*).

Intraoperative angiography was performed after these maneuvers to demonstrate vessel occlusion (Figure 2A). Additionally, intraoperative ICG angiography demonstrated no detectable blood flow to the MCA territory distal to the lenticulostriate vessels and proximal to the collateral MCA vessels (Figure 2C). The temporary clips were removed after 1 hour, and the craniectomy was closed.

Four dogs underwent and survived this procedure. MRI taken 1 day after the procedure demonstrated a territorial MCA infarct that had spared the deep gray matter (Figure 1D). Although these procedures had been performed on separate days, the stroke volume for these dogs was remarkably consistent, measuring 9.9, 8.5, 9.9, and 8.2 cm^3, with an average stroke volume of 9.13 $\pm$ 0.90 cm^3 (Table 1). The results from the neurologic examinations were quantified using the CSCARS. Three dogs underwent the CSCARS on postoperative day 1. The CSCARS scores were 25, 25, and 30, with an average neurologic examination score of 26.7 $\pm$ 2.9. The results from follow-up neurologic examinations were available for 2 of these dogs at 1 week. Both dogs had shown modest improvement in neurologic examination, with a CSCARS score of 23 (initially 25) and 29 (initially 25), respectively (average score, 26 $\pm$ 4.24; Table 1). The missing neurologic examination was because of the unavailability of the veterinary specialist performing the CSCARS assessment.

At 2 weeks, the dogs were sacrificed. The brain was collected at necropsy and fixed in formalin. Sectioning was performed, followed by hematoxylin and eosin staining. The regions of stroke were characterized on pathological examination and closely corresponded to the regions of T2-weighted hyperintensity on MRI (Figures 3 and 4).

DISCUSSION

Development of a consistent and reproducible territorial stroke model in gyrencephalic animal models remains a critical need in stroke investigations. The canine models of stroke have several advantages in this regard.[4,18-21] However, the available data have suggested that anatomic variations in the perfusion contribution from the collateral circulation have contributed to the significant variability in the stroke volume generated.[22] In the present study,

Figure 1. Schematic depicting the stroke model. Schematic drawings of the canine circle of Willis depicting vessel occlusion during stroke surgery and accompanying T2-weighted axial magnetic resonance imaging (MRI) scans obtained 1 day after stroke (area of stroke indicated by *white dotted circle*). In the schematic drawings, vessel clips are shown in *brown* and had been applied to the respective vessel for 1 hour. (**A**) Illustration showing temporary vessel clip applied to the proximal middle cerebral artery (MCA) and (**A'**) MRI scan depicting no evidence of stroke. (**B**) Temporary vessel clips applied to the proximal MCA, anterior cerebral artery (ACA), and ophthalmic artery (OA) between the ACA and MCA, and (**B'**) MRI scan depicting no evidence of stroke. (**C**) Temporary vessel clips applied to the proximal MCA, ACA, OA, and posterior cerebral artery (PCA), and MRI scans depicting (**C'**) a large stroke in the right frontal and temporal regions and in the basal ganglia (*asterisk*) in 1 dog and (**C''**) a very small stroke in a separate dog. (**D**) Temporary vessel clips applied to the MCA, distal to the lenticulostriate vessels, ACA, and OA. The distal collateral connections to the MCA had been coagulated (*yellow bands*). (**D',D''**) MRI scans depicting stroke in the right frontal and temporal regions with sparing of the basal ganglia in 2 separate dogs. *n*, total number of dogs in which the respective procedure had been performed.

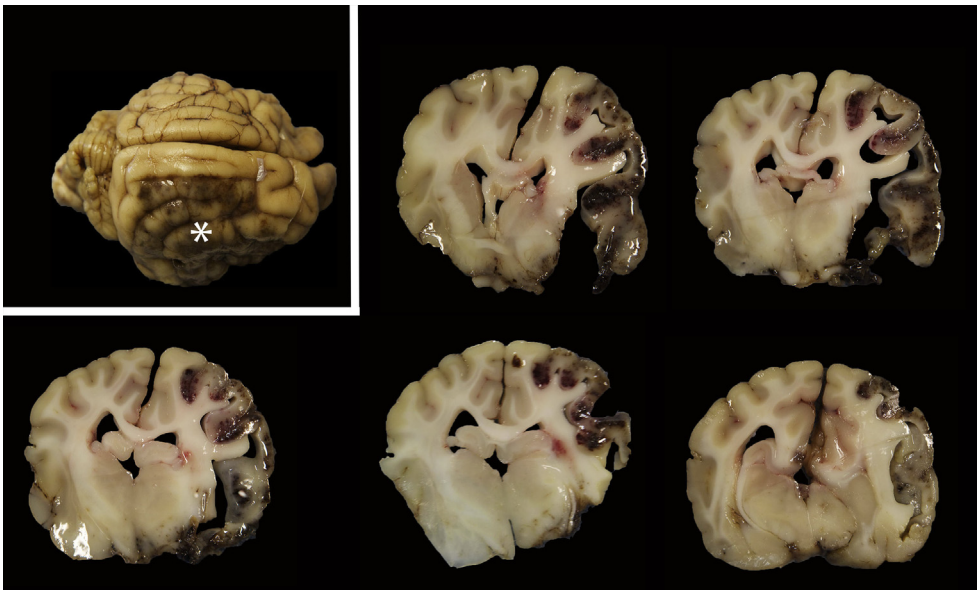

Figure 3. Anatomic images depicting area of infarction. Canine brain gross pathology specimen obtained 1 day after stroke. (**Inset**) Whole brain viewed from the side with area of infarction evident on the cortical surface (*asterisk*). Coronal sections depicting the area of infarction on the left side of the brain.

we have described a neurosurgical approach that leverages the familiarity and skill sets of neurosurgeons with the cerebral vasculature and skull base to create consistent, territorial MCA stroke volumes. The model involves a craniectomy approach to expose the ICA, ACA, OA, and MCA. The distal MCA pial collateral vessels are then cauterized, and temporary clips are applied to the MCA (distal to the lenticulostriate vessels), OA, and ACA for 1 hour. The major advantages of the model include

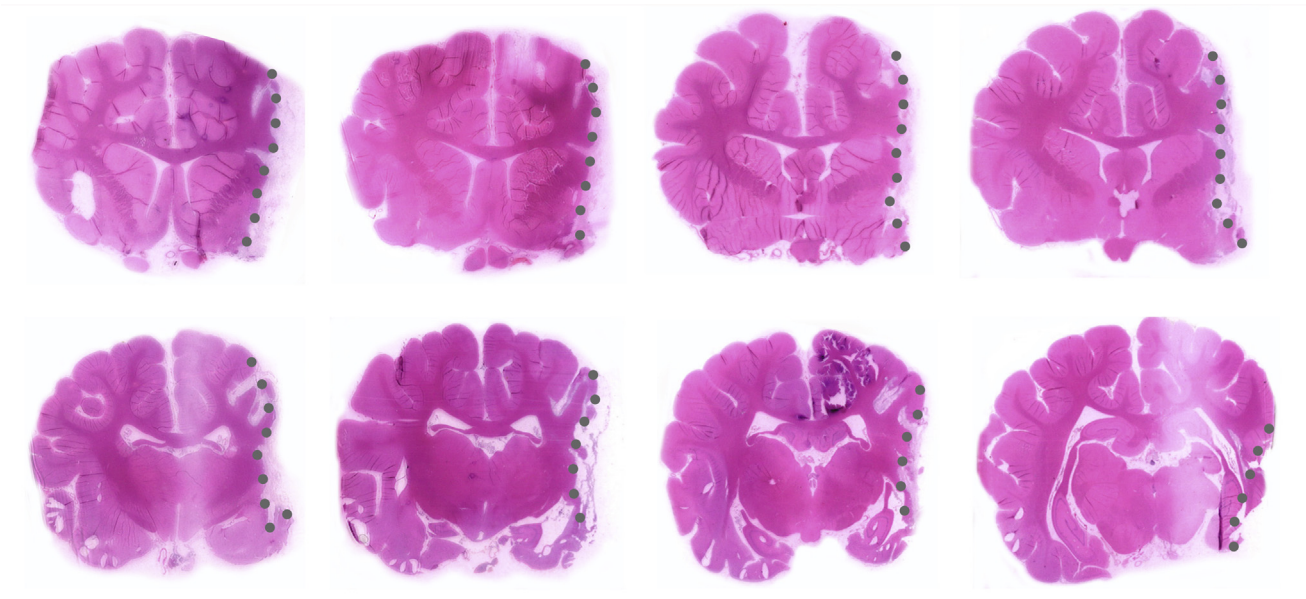

Figure 4. Histologic images depicting area of infarction. Coronal sections of canine brain obtained 14 days after stroke. Hematoxylin and eosin staining depicting normal white and gray matter. The area of infarction is highlighted (*dotted line*). Due to processing, most of the friable tissue in the area of stroke had sloughed off during the sectioning process.

the following: 1) relative ease of surgery for neurosurgical practitioners; 2) survival of the beagle with consistent neurologic deficits after undergoing the procedure; 3) a consistent volume of territorial MCA stroke; 4) a large-size craniectomy to accommodate stroke-related malignant edema; and 5) opportunities for cerebral angiographic studies.

Canine stroke models have been developed using both endovascular and surgical approaches.[4,12,13,16-18,20-29] Although either approach can successfully induce stroke, both approaches have been associated with significant inconsistencies in the stroke volume owing to the extensive pial collateral circulation present in dogs.[22] Most surgical approaches have isolated the MCA through the orbit, which requires eye enucleation. This is morbid for the dogs and can complicate the neurologic assessment after stroke.[29] Using the endovascular approaches, the tortuosity of the ICA at the skull base often precludes consistent occlusion of the MCA.[4,17,22,23] Moreover, such endovascular approaches have been associated with high rates of vessel perforation.[22] The approaches to the MCA through the basilar artery are often accompanied by severe vasospasm of these vessels, increasing the risk of fatality.[4,22] Additionally, when large strokes are generated by endovascular approaches, significant brain edema will ensure, which will be associated with high mortality because of the increased intracranial pressure.[4,28] The advantages of our model are evident when considered in these contexts.

Although we were encouraged by the consistency of our beagle stroke model, we also recognize that the present study was limited by the number of the dogs tested. Recognizing this limitation, however, the cost and efforts of our study were on par or exceeded those of murine stroke studies involving hundreds of animal subjects. This observation underlies the fundamental challenge in gyrencephalic animal stroke models and highlight the importance of our efforts as a proof-of-principle study. Two additional issues involve our limited understanding of the pathophysiology of the canine stroke model and the absence of data from female beagles. Efforts are currently underway to validate this model in female beagles and obtain more detailed immunohistologic characterization of the underlying pathophysiologic processes in this canine stroke model. These efforts should afford opportunities for more rationale usage of this model in future stroke research and allow us to meet the mandates of the National Institutes of Health for the "testing of potential therapies in multiple species."

CONCLUSION

We have developed a canine stroke model using a skull-base surgical approach that creates consistent and reproducible MCA territorial stroke. The model could aid in future preclinical stroke studies focused on testing cellular therapies, neuroplasticity, and neuroregenerative therapies for stroke.

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